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Review

Monthly Focus: Central & Peripheral Nervous Systems

Recent progress in 5-HT$_6$ receptor antagonists for the treatment of CNS diseases

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In light of the barrage of recent reviews on 5-HT$_6$ receptor antagonists, this article highlights and reviews the research advances published in patent literature between January 1998 and December 2001. The article is supplemented with selected references on design, synthesis and development of novel 5-HT$_6$ agents to treat CNS diseases and to study and understand their mechanism and pathophysiology. Emphasis is given to recent advances in the possible involvement of 5-HT$_6$ serotonergic agents in the treatment of schizophrenia and depression. By no means has any attempt been made to exhaustively review the literature but rather, primary references along with citations to recent literature reviews have been included in each section.

Keywords: 5-HT$_6$, agonists, antagonists, cognitive, depression, dysfunction, potency, schizophrenia, selectivity, side effects

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1. Introduction

The discovery of several antipsychotic agents (notably clozapine 1, olanzapine 2 and seroquel 3) and antidepressants (clomipramine 4, amitriptyline 5, doxepin 6 and nortryptyline 7) as highly potent 5-HT$_6$ receptor antagonists [1-3] has led to acceleration in research efforts toward finding more potent and selective 5-HT$_6$ receptor antagonists. The high 5-HT$_6$ receptor affinity of these therapeutically important antipsychotics and antidepressants seems to suggest a possible role for this receptor, and hence 5-HT$_6$ antagonism, in the treatment of schizophrenia and depression. Considerable advances have been made in the research and discovery of more potent and selective 5-HT$_6$ receptor antagonists since the patent literature was last reviewed in 1998 [4]. More intriguing is the increased understanding of the mechanism and pathophysiology of 5-HT$_6$ antagonism in the treatment of CNS diseases. The importance of 5-HT$_6$ antagonists is manifested by the growing numbers of patents filed and scientific papers published in recent years. These efforts have yielded highly potent and selective ligands to target relevant receptor subtypes in the treatment of CNS diseases.

5-HT (serotonin), a key neurotransmitter of the CNS and PNS, has been implicated in a variety of sensory, motor and behavioural processes [1]. Diverse effects of this neurotransmitter are related to the extensive projections of serotonergic neurons throughout the brain and large number of distinct serotonin receptor subtypes. At least 14 distinct serotonin receptor subtypes are expressed in the mammalian CNS [8,9]. These receptors have been classified into seven main families: 5-HT$_{1-7}$. The 5-HT$_1$ family comprises subtypes 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5-HT$_{1F}$; the 5-HT$_2$ family consists of subtypes 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ and the 5-HT$_5$ family consists of subtypes 5-HT$_{5A}$ and 5-HT$_{5B}$. During the last four years, ~ 90% of patent applications citing CNS diseases claim serotonergic

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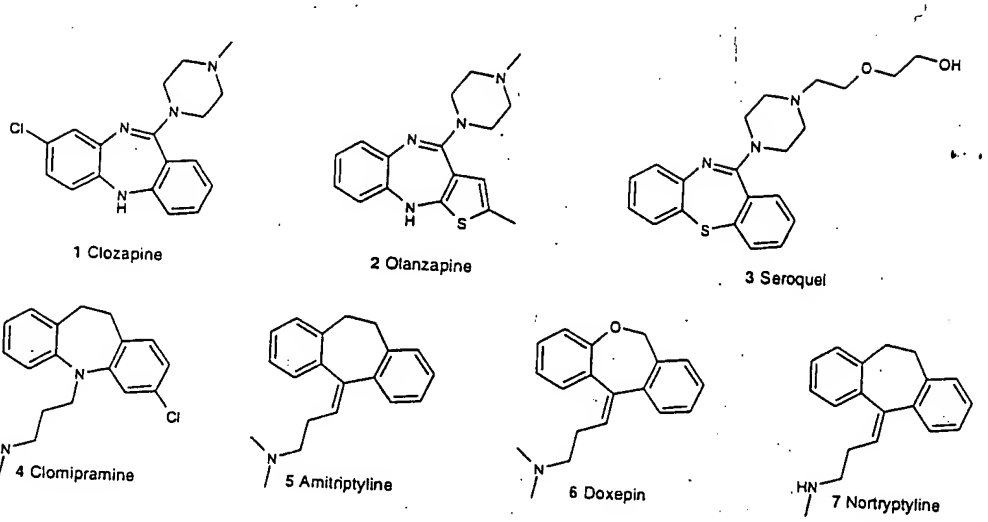


agents. This section will cover 5-HT$_6$ serotonergic agents, particularly 5-HT$_6$ receptor antagonists and their implication in the treatment of CNS disorders.

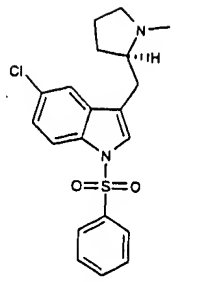
The human 5-HT$_6$ receptor, one of the most recently cloned serotonergic receptors, is a 440-amino acid polypeptide with seven transmembrane spanning domains, typical of the G-protein-coupled receptors [9]. Within the transmembrane region, the human 5-HT$_6$ receptor shows ~ 30 – 40% homology to other human 5-HT receptors and is positively coupled to adenylyl cyclase activity. The prominent localisation of 5-HT$_6$ receptor mRNA in the nucleus accumbens, striatum, olfactory tubercule, substantia nigra and hippocampus of the brain [10], together with high affinity of the 5-HT$_6$ receptor for several therapeutically important antipsychotics and antidepressants, suggest a possible role for this receptor in the treatment of schizophrenia and depression. In fact, the prototypic, atypical antipsychotic agent clozapine exhibits greater affinity for the 5-HT$_6$ receptor than for any other receptor subtype [11,12]. The 5-HT$_6$ receptor was identified using molecular biology techniques without prior knowledge of its physiological function or pharmacology. Subsequently, a number of different techniques have been used to identify the function of the 5-HT$_6$ receptor, including experiments with antisense oligonucleotides, transgenic animals and the identification of selective antagonists for the receptor using high-throughput screening and classical medicinal chemistry.

Although the 5-HT$_6$ receptor has a distinct pharmacological profile, *in vivo* investigation of receptor function has been hindered by the lack of selective agonists and antagonists. Recent experiments demonstrated that chronic intracerebroventricular treatment with an antisense oligonucleotide directed at 5-HT$_6$ receptor mRNA, elicited a behavioural syndrome in rats consisting of yawning, stretching and chewing. This syndrome in the antisense-treated rats was dose-dependently antagonised by atropine (a muscarinic antagonist), implicating the 5-HT$_6$ receptor in the control of cholinergic neurotransmission. Therefore, 5-HT$_6$ receptor antagonists may be useful for the treatment of memory dysfunction [13,14].

2. Potential therapeutic indications for the 5-HT$_6$ receptor

More recently, the development of newly created probes, such as selective antibodies, selective antagonists, transgenic animals and genetic linkage studies, has been used to help further understand the possible therapeutic functions of the 5-HT$_6$ receptor. Over the last three years there has been substantial progress in the elucidation of this newly discovered receptor. The prominent localisation of the 5-HT$_6$ receptor in the limbic and cortical regions and the discovery that the atypical antipsychotic drugs have higher affinity for the 5-HT$_6$ receptor suggest that this new receptor might play a role in the pathophysiology of CNS diseases, such as schizophrenia and bipolar affective disorder. This finding encouraged a search for more potent and highly selective 5-HT$_6$ antagonists as a new class of antipsychotics. Few compounds achieving high potency and selectivity are now available for proof of concept studies. These include ALX1161 8, ALX1175 9 (NPS), SB-271046 10, SB-357134 11 (GlaxoSmithKline), Ro 04-6790 12 and Ro 63-0563 13 (Hoffmann-La Roche).

Recently, a variety of studies have shown that the 5-HT$_6$ receptor is targeted by several atypical antipsychotics, including clozapine and olanzapine [15]. As a result, this target was hypothesised to contribute to their therapeutic actions. In recent studies, a silent polymorphism described in the 5-HT$_6$ receptor gene 267-C/T has been reported to be associated



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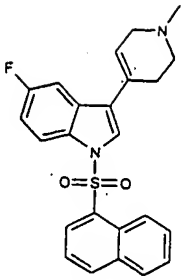

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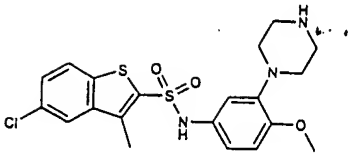

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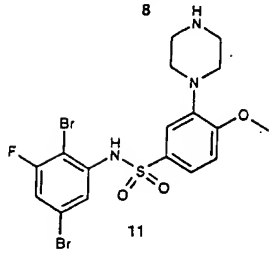

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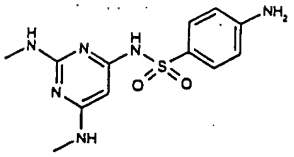

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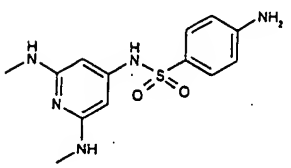

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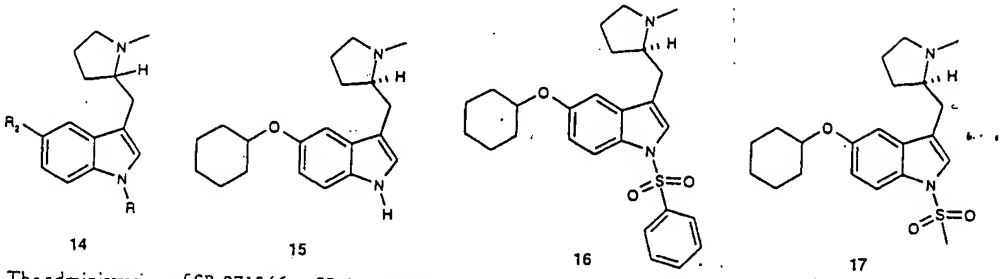
with schizophrenia and Alzheimer's disease (AD) [16]. In addition, association between the good response to clozapine and the T/T genotype has been reported. To test this hypothesis, this polymorphism was genotyped in two independent samples of clozapine-treated patients and a sample of olanzapine-treated patients, including responders and non-responders. Preliminary results of the olanzapine study show no association between this polymorphism and olanzapine response, although the results follow the same trend as that of clozapine. A stratified analysis of both clozapine samples showed a slight association between the polymorphism and clozapine response genotypes (p = 0.05) and alleles (p = 0.02). It has been concluded that these results provide further evidence to suggest that the 5-HT_6 267-C/T polymorphism may contribute to the prediction of clozapine response [15]. In a study, Vogt *et al.* [16] performed a systematic mutation scan of the complete coding region and splice junction of the 5-HT_6 receptor gene to explore the contribution of this gene to the development of bipolar affective disorder and schizophrenia. Investigating 137 unrelated individuals (including 45 bipolar affective patients, 46 schizophrenic patients and 46 unrelated controls) and comparing frequencies between patients and controls, the authors claimed a significant over-representation of the 267C allele among bipolar patients (p = 0.023 not corrected for multiple testing). This finding was followed up in independent sample of 105 bipolar family trios using a family based association design. Fifty-one transmissions could be examined and alleles 267C and 267T were transmitted to the affected offspring in 30 and 21 cases, respectively. The authors claimed that these preliminary data suggest that bipolar affective disorder may be associated with variation in the 5-HT_6 genes and it will be important to extend the present analysis to larger samples [16].

The advent of pharmacologically selective 5-HT_6 receptor ligands has allowed experimental confirmation of previous experimental outcomes from antisense studies supporting the role of the 5-HT_6 receptor in the control of central cholinergic function. Cholinergic involvement in mediating 5-HT_6 receptor function was also suggested in a behavioural study of rats unilaterally-lesioned with 6-hydroxydopamine. Unlike L-DOPA or amphetamine, Ro 04-6790 did not cause rotational behaviour in these rats. However, Ro 04-6790 did attenuate scopolamine and atropine-induced circling behaviour in a dose related manner. Reproduction of these effects using SB-271046 was not successful, suggesting that the exact nature of the 5-HT_6 receptor/cholinergic interaction still demands further resolution. In a separate study, the acetylcholinesterase inhibitor, physostigmine elicited yawning in rats, which was modestly potentiated by SB-271046. There is clearly a need to further investigate the involvement of the 5-HT_6 receptor in this behavioural syndrome (yawning) using a wider range of antagonists from different structural classes. Nevertheless, recent co-localisation studies in rat brain seem to suggest that the 5-HT_6 receptor regulation of central cholinergic transmission is not via direct dis-inhibition of central cholinergic neurons *per se*, but through dis-inhibition of GABAergic neurons [17-19].

Despite the conflicting results obtained in the behavioural syndrome studies (thought to be mediated by 5-HT_6 receptors); there are some areas of concurrence in the study of 5-HT_6 receptor pharmacology. The cognition-enhancing properties of SB-271046 and SB-357134 were investigated in the Morris water maze test of spatial learning and memory in



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rats. The administration of SB-271046 or SB-357134 had no effect on learning *per se*, however, both compounds produced a significant improvement in retention of a previously learned task. By contrast, the acetylcholinesterase inhibitor, donepezil (Aricept™, Eisai) had no effect in this task, thereby demonstrating that 5-HT$_6$ receptor antagonism may be involved in cognitive function. Similarly, Ro 04-6790 or 5-HT$_6$ antisense oligonucleotide enhanced retention of the learned platform position in the Morris water maze, without affecting acquisition. These preliminary results suggest that 5-HT$_6$ receptor antagonists may play a role in the treatment of cognitive dysfunction [20-23].

Interestingly, a recent *in vivo* microdialysis study demonstrated an interaction between the 5-HT$_6$ receptor system and glutamate. The 5-HT$_6$ receptor antagonist SB-271046, was found to increase frontal cortical extracellular glutamate and aspartate levels in a tetrodotoxin-sensitive manner. Levels of neurotransmitters, such as noradrenaline, dopamine and serotonin were unaffected in the frontal cortex. In contrast, none of the neurotransmitter levels in the striatum were affected. This 5-HT$_6$ receptor antagonist effect on cortical glutamate levels is of particular significance in view of the hypothesis of glutamate hypofunction in schizophrenia. In addition, these data may also provide clues to the mechanism of action of 5-HT$_6$ antagonists in the facilitation of cognitive function [24-26].

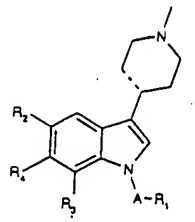
Further involvement of 5-HT$_6$ receptor antagonists in seizures (anticonvulsant) [27-29], depression and anxiety is presently under evaluation. As new and selective 5-HT$_6$ receptor antagonist tools become more widely available, it is now possible to evaluate the pharmacology of these compounds with respect to direct interaction with the 5-HT$_6$ receptor and therefore determine the therapeutic potential of this novel target.

3. Emerging 5-HT$_6$ receptor antagonists

Much research is in progress to find new and more effective medication or therapies for CNS disorders. There is growing interest in this exciting field as evidenced by the active participation of essentially all major pharmaceutical companies and the number of related patents. Preliminary studies suggested that clozapine's exceptional properties, especially its efficacy in treatment-resistant patients and lack of extrapyramidal side effects, might possibly arise from selective antagonism of the 5-HT$_6$ receptor rather than dopamine D2 and D4 receptors. These findings have ignited the search for more potent and selective 5-HT$_6$ receptor antagonists as a new class of safer and more efficacious antipsychotics. Recent drug discovery efforts in the serotonin field have clearly been oriented towards the development of more selective ligands that discriminate between 5-HT$_6$ and other serotonin receptor subtypes. Considerable efforts are currently focusing on the development of novel and selective 5-HT$_6$ receptor antagonists, notably from companies such as NPS Pharmaceuticals, GlaxoSmithKline, Hoffmann-La Roche, Merck and Pharmacia & Upjohn.

3.1 NPS Pharmaceuticals

NPS researchers have reported the discovery of several series of highly potent and selective human 5-HT$_6$ receptor antagonists. The first patent from this group was published in 1999, describing a novel series of 3-pyrrolidin-1-substituted indole derivatives represented by the general formula 14 for the treatment of schizophrenia and other CNS diseases [30,101]. A wide range of substituted N-benzoyl and sulfonyl indoles were prepared by parallel synthesis and many of these compounds were found to demonstrate excellent binding profiles. The structure-affinity relationship in this series shows that 5-cyclohexyloxy-1H-indole derivative 15, was a potent 5-HT$_6$ receptor ligand (K_i = 0.93 nM) and exhibited full agonism (IC_{50} = 16.5 nM). However, the introduction of the phenylsulfonyl group at the nitrogen of the indole skeleton led to derivative 16, with reversal of functional activity from agonist to antagonist (K_i = 0.87 nM and IC_{50} = 53.8 nM). The replacement of the phenyl moiety in derivative 16 with a methyl group led to its analogue 17 with 267-fold decrease in receptor affinity (K_i = 233 nM). A range of lipophilic aromatic sulfonyl groups were tolerated, giving compounds with high affinity for the human 5-HT$_6$ receptor. On the other hand, replacement of the cylohexyloxy group of 16 with a simple chloro group afforded the lead compound (ALX1161) in this series. ALX1161 exhibited excellent human 5-HT$_6$ receptor binding (K_i = 1.4 nM) and demonstrated good antagonistic activity (IC_{50} = 8.5 ± 1.1 nM) in a cAMP functional assay. Furthermore, ALX1161 displayed > 100-fold selectivity over a battery of 40 other receptors and binding sites, including serotonergic and dopaminergic receptors. Upon pharmacokinetic analysis in rat, ALX1161 displayed an average whole brain versus plasma ratio of 23.4 ± 2.8 following i.v.



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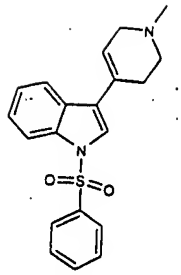

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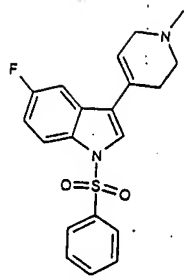

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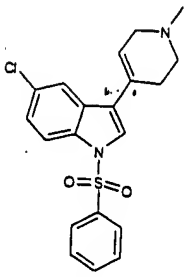

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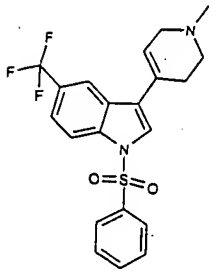

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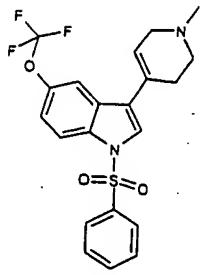

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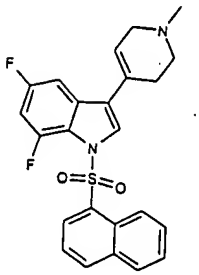

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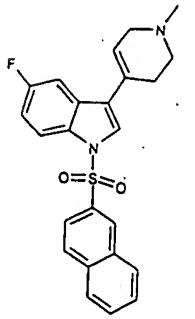

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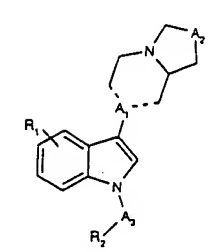
administration, $t_{½}$ = 91 ± 6 min, steady-state volume of distribution = 1.2 ± 0.2 l/kg following i.v. bolus administration and oral bioavailability = 17 ± 9% following po. administration in male Sprague-Dawley rats.

Similarly, in another patent application the same group claimed a series of novel piperidine indole derivatives represented by the general formula 18 and having human 5-HT_6 receptor affinity. This class of compounds was claimed for the treatment of a variety of CNS diseases, including AD, Huntington's chorea, schizophrenia, cognitive disorder and manic depression [31,102]. SAR studies show that the nature of substitution of the indole moiety at the 5-position does not affect the 5-HT_6 binding activity. In fact, keeping the substitution of the indole nitrogen fixed as the phenylsulfonyl group, substitution at the 5-position of the indole skeleton with R_2 (R_2 groups such as a hydrogen 19, fluoro 20, chloro 21, trifluoromethyl 22, or trifluoromethoxy 23), led to approximately the same 5-HT_6 receptor activity profiles (K_i = 3.1 nM, 2.5 nM, 3.0 nM, 2.2 nM and 4.9 nM, respectively). In contrast, the disubstituted indole derivatives such as, 5,7-difluoro 1-(naphthylsulfonyl) indole 24 led to a decrease in the human 5-HT_6 receptor affinity compared to its mono-substituted analogue 9 (ALX1175) (K_i = 1 and 21 nM, respectively). Furthermore, the 1-naphthylsulfonyl derivative 9 was about 20-fold more potent at the human 5-HT_6 receptor compared to the corresponding 2-naphthylsulfonyl analogue 25 (K_i = 1 nM vs 19.5 nM respectively). In the functional adenylyl cyclase assay, the most potent compound, ALX1175 was found to be a competitive antagonist (IC_{50} = 23.7 ± 4.2 nM) with > 100-fold selectivity over a number of other key receptors. ALX-1175 has good CNS penetration (whole brain vs. plasma ratio of 43.4 ± 5.9 following iv. administration), $t_{½}$ = 60 min and oral bioavailability = 19 ± 0.2%.

Replacement of the piperidine ring in ALX-1175 series with bicyclic-piperidine and bicyclic-piperazine moieties was the subject of another patent application by the NPS research group. The general structure claimed in this patent was exemplified by 26. These compounds were stated to be 5-HT_6 receptor antagonists useful for the treatment of psychosis, schizophrenia, depression, manic depression, neurological and memory disturbances, Parkinson's disease (PD) and amyotrophic lateral sclerosis. Over 50 analogues were exemplified. The compound, 5-fluoro-3-[(8a-*R*,*S*)-1,2,3,5,8,8a-hexahydroindolizin-7-yl]-1-phenylsulfonylindole 27 and their analogues 6,5-bicyclic-piperazine 28 and 6,6-bicyclic-piperazine 29 were among the specific compounds claimed. The binding affinity of these compounds and their functional activity at the human 5-HT_6 receptor, were assessed *in vitro*. Compound 27 showed > 90% inhibition (at 1 μM) of radioligand binding at the human 5-HT_6 receptor and < 10% binding activity at 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors. Compound 27 was also claimed to antagonise human 5-HT_6 receptor mediated cAMP accumulation in HEK-293 cells [103]. In addition, the NPS chemists designed and synthesised novel azaindole derivatives, as a second generation of the previously mentioned series. The compound 3-(1,2,3,5,8,8a-hexahydroindolizin-7-yl)-1-naph-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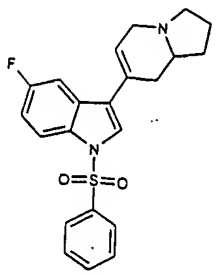
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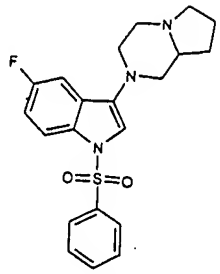
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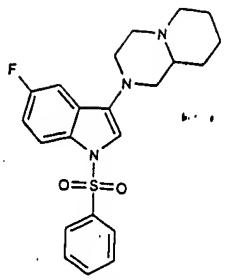
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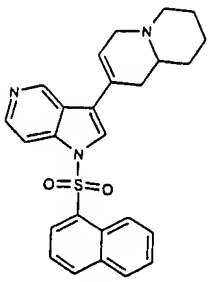
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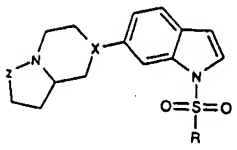
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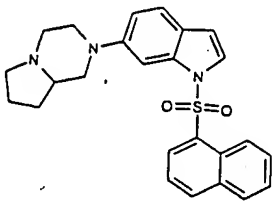
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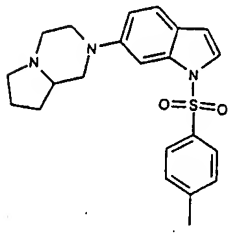
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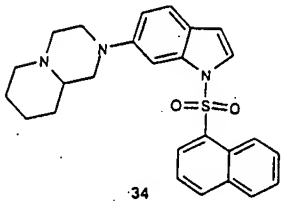
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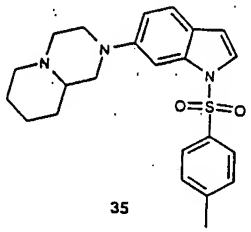
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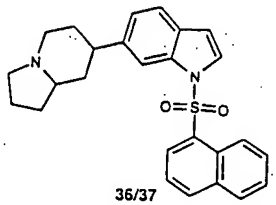
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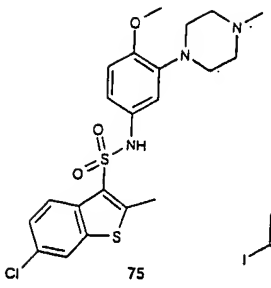
thelen-1-ylsulfonyl)-5-azaindole 30 was one of the compounds specifically claimed. Compound 30 showed > 95% inhibition (at 1 μM) of the human 5-HT$_6$ receptor and is one of four compounds shown to reverse the stimulation of adenylyl cyclase by 5-HT in HEK-293 cells expressing the human 5-HT$_6$ receptor [104]. The same team elegantly designed highly potent and selective human 5-HT$_6$ receptor ligands, by moving the bicyclic-piperidine and bicyclic-piperazine from the 3-position to the 6-position of the indole skeleton. This novel series was exemplified by the 6-bicyclopiperazinyl-1-arylsulphonylindoles and the 6-bicyclopiperidinyl-1-arylsulphonylindoles having the general structure 31. In general, all of the compounds tested above were found to be very potent at the human 5-HT$_6$ receptor with K_i values < 10 nM. In the case of the bicyclopiperazine derivatives, the 6,5-bicyclopiperazinyl-1-arylsulfonylindole analogues were more potent than the corresponding 6,6-bicyclopiperazinyl-1-arylsulfonylindole. For example, compound 32 where R is a 1-naphthyl group, binds to the human 5-HT$_6$ receptor with a K_i value of 0.19 nM. The analogue 33 where the R group is p-tolyl has a K_i value of 3.3 nM. These analogues have a greater affinity for the human 5-HT$_6$ receptor than their bicyclic homologues 34 and 35, with K_i values of 2 nM and 4.7 nM, respectively. Of the monocyclic and bicyclic aromatic sulfonyl groups studied, the lipophilic bicyclic substituent, such as the 1-naphthyl group, was beneficial to human 5-HT$_6$ receptor activity. The rapid optimisation of the aryl sulphonyl groups (1-naphthyl group favoured) along with the realisation that the 6,5-bicyclopiperazine-systems were generally more potent than the corresponding 6,6-bicyclopiperazine homologue, prompted the examination of the 6,5-bicyclopiperidine analogues 36 and 37. Both isomers 36 and 37 (designated as the less polar and the most polar, respectively) demonstrated sub-nanomolar human 5-HT$_6$ receptor affinity (K_i = 0.8 and 0.7 nM, respectively). In addition, the 6,5-bicyclopiperidine analogues 38 and 39 maintained good 5-HT$_6$ receptor activity (K_i = 4.7 and 1.2 nM, respectively) further indicating the degree of structural variations allowed without compromising 5-HT$_6$ receptor affinity. In the functional adenylyl cyclase assay, the most potent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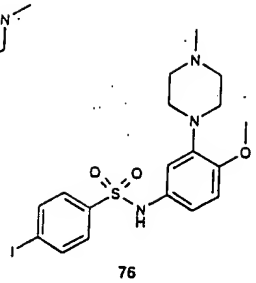
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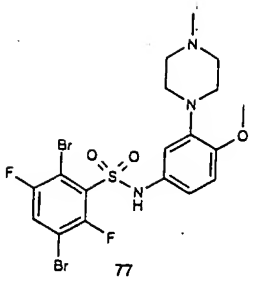
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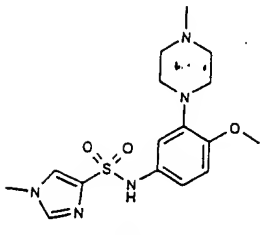
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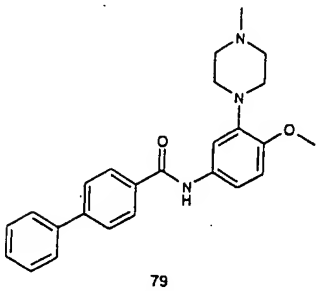
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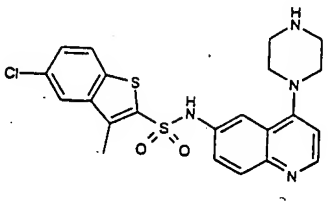
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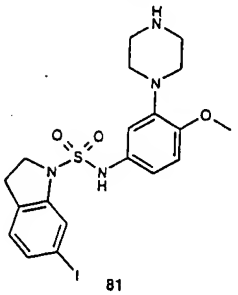
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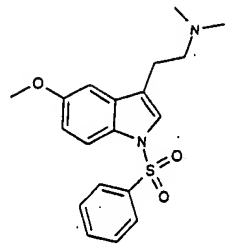
mide 79 was among the 13 compounds specifically claimed. Although, no biological data were disclosed, these compounds were stated to show pK_i values of > 7.0 [114]. The outcome of these medicinal chemistry efforts led to the 5-chloro-3-methylbenzothiophene 73 as the optimal compound for further evaluation. Pharmacokinetic studies of compound 73 at steady-state in rat following a 16 h infusion demonstrated that, it was moderately brain penetrant (18%) and had relatively low blood clearance compared to 73 (12.5 ml/min/kg vs. 60 ml/min/kg). However, in rats, compound 73 was metabolically labile, undergoing N-demethylation to the corresponding NH-piperazine analogue SB-271046. The receptor binding profile of SB-271046 showed a slightly reduced human 5-HT_6 affinity relative to the N-methylpiperazine derivative 73 (pK_i = 8.9). SB-271046 was shown to be a competitive antagonist with a pA_2 value of 8.7. Furthermore, SB-271046 was found to be highly selective (> 200-fold) against a battery of > 50 receptors, enzymes, or ion channels. Pharmacokinetic studies demonstrated this metabolite to be moderately brain penetrant (10%), subject to low blood clearance (7.7 ml/min/kg) with a good half-life in rats (4.8 ± 0.1 h) and had excellent oral bioavailability (F = 80%). The replacement of the (4-methoxy-1-piperazinyl)phenyl moiety in 73 by quinoline substituted at the 4-position 80, yielded a similar binding affinity for the 5-HT_6 receptor (pK_i = 8.7) [47]. In an attempt to increase the brain penetration of these compounds, further SAR development in the piperazine-benzenesulfonamide series (i.e., replacing sulfonamide NH with more lipophilic groups) led to the discovery of conformationally restricted indoline analogues 81 and 82 with high affinity for the 5-HT_6 receptor (pK_i = 9.5 and 8.4, respectively). Similar conformational analogues, such as the tetrahydroquinoline and isoquinoline derivatives 83 and 84 also possessed good 5-HT_6 receptor affinity (pK_i = 9.5 and 9.3, respectively). However, compounds from this series, such as 82 and 84, had *in vivo* clearance in rats ≥ liver blood-flow [48,115]. These efforts also led to discovery of the [^{125}I] radiolabelled compound 4-iodo-*N*-[4-methoxy-3-(4-methypiperazin-1-yl)phenyl]benzenesulfonamide 76 ([^{125}I]SB-258585). In addition to [^{3}H]-Ro 63-0563, [^{125}I]SB-258585 is an important tool for helping the scientific community in further understanding the therapeutic benefits of the 5-HT_6 receptor [39,49].

3.4 Merck

Through internal screening of their sample collection, Merck recently reported their discovery that N-(arylsulfonyl)indole derivatives were potent, selective human 5-HT_6 receptor ligands [50,116]. This finding was previously reported by Glennon *et al.* in collaboration with NPS pharmaceuticals. One lead candidate, compound 46, was found by both groups to be a potent and selective human 5-HT_6 receptor antagonist. Similar results were also obtained upon profiling this compound against a number of serotonin and dopamine receptors, demonstrating that 46 exhibited considerable affinity for the 5-HT_2 receptor (K_i at 5-HT_2 was 65 nM). However, the Merck researchers then determined whether compound 46 penetrated the brain and interacted with the serotonin receptors by using head twitch response elicited by 5-HT_2 receptor agonist, such as mescaline and DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane], that is selectively blocked by selective 5-HT_2 receptor antagonists. In this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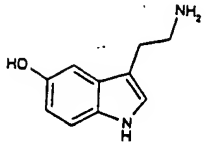
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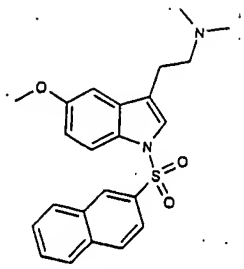
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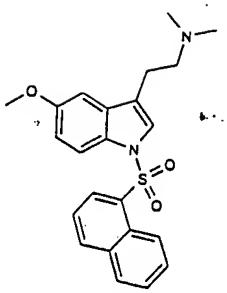
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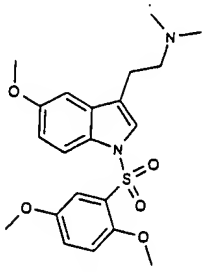
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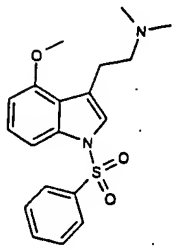
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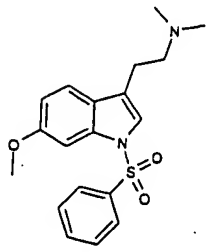
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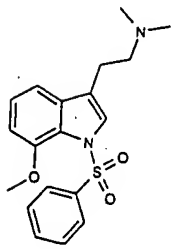
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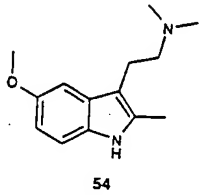
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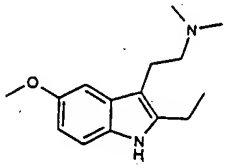
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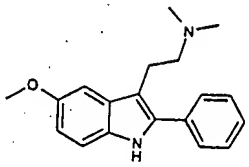
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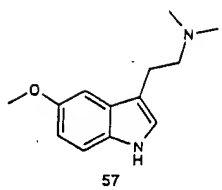
5-HT$_{2A}$ and rat 5-HT$_{2C}$ receptors (K_i = 130 ± 65 nM and K_i = 23 ± 5 nM, respectively). Compound 46, the parent member of the series, was examined both as an agonist and as an antagonist in an adenylyl cyclase assay. Compound 46 lacked agonist character at a concentration of 10,000 nM. However, this single concentration completely blocked 5-HT-stimulated adenylyl cyclase activity. Subsequent testing showed that compound 46 produced inhibition of adenylyl cyclase activity in a dose-dependent manner (pA_2 = 8.88 ± 0.2 nM) [34]. Glennon *et al.* also studied several 2-alkyl-5-methoxytryptamine analogues. The SAR showed that the 5-HT$_6$ receptor accommodated small alkyl substituents at the 2-position of the indole skeleton and that the resulting compounds can bind with affinities comparable to that of serotonin 47. Analogues 2-methyl-5-methoxy-*N*,*N*-dimethyltryptamine 54, 2-ethyl-5-methoxy-*N*,*N*-dimethyltryptamine 55 and 2-phenyl-5-methoxy-*N*,*N*-dimethyltryptamine 56 were synthesised for examination of detailed binding profiles. All three derivatives were examined at > 30 different receptor populations, including serotonergic and dopaminergic receptors, and produced < 50% inhibition of binding at a concentration of 1 µM at the majority of these receptors. Compounds 55 and 56 bind to human 5-HT$_6$ receptor with comparable affinity (K_i = 16 ± 4 and 20 ± 5 nM, respectively) and with affinity similar to that of clozapine (K_i = 10 ± 3). The three compounds (54, 55 and 56) were assessed for their ability to activate adenylyl cyclase. While compounds 54 and 55 behaved as full agonists (K_{act} = 9.7 ± 5.0 and 3.6 ± 51.3 nM, respectively) relative to 5-HT (K_{act} = 5.0 ± 3.0 nM), compound 56 showed no agonist activity. Another idea was to subsequently incorporate a 2-alkyl substituent into the N1-substituted analogue that retained good 5-HT$_6$ receptor affinity. N1-methylation of 5-methoxy-*N*,*N*-dimethyltryptamine 57 (K_i = 78 nM) decreased 5-HT$_6$ receptor affinity of the resulting compound by > 6-fold (58, K_i = 510 nM). Homologation of the N1- methyl group to an ethyl group (59, K_i = 240 nM) or n-propyl group (60, K_i = 200 nM) increased the affinity for the 5-HT$_6$ receptor by 2-fold. However, these compounds (58, 59 and 60) did not bind as successfully as compound 57. Nevertheless, the corresponding isopropyl derivative 61 (K_i = 130) resulted in a slight enhancement in 5-HT$_6$ receptor affinity compared to the other alkyl analogues. However, none of these compounds displayed significantly enhanced affinity. Compound 62, which may be viewed as cyclic 1,2-disubstituted analogue of compound 55, was also prepared for evaluation and found to bind with reduced affinity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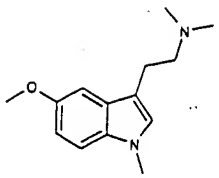
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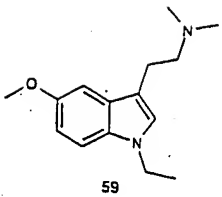
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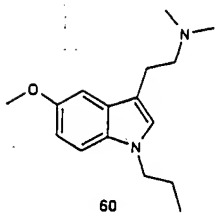
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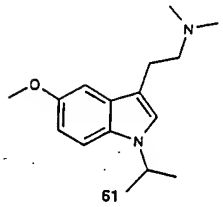
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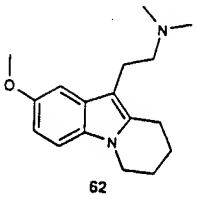
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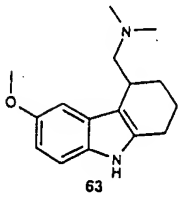
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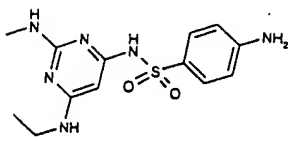
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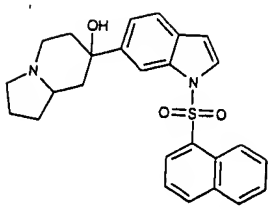
(K_i = 1030 nM). Finally, the 2-ethyl group in 57 was tethered to afford compound 63. Compound 63 was found to bind to the 5-HT_6 receptor with ~ 3-fold lower affinity than 55 (K_i = 168 nM) [35-37,106].

3.2 Hoffmann-La Roche

Hoffmann-La Roche researchers were the first to identify potent and selective 5-HT_6 receptor antagonists exemplified by the two lead compounds 4-amino-*N*-(2,6-*bis*-methylamino-pyrimidin-4-yl)-benzene sulfonamide Ro 04-6790 and 4-amino-*N*-(2,6-*bis*-methylamino-pyridin-4-yl)-benzene sulfonamide Ro 63-0563. These two compounds were found to have reasonable affinity for the rat 5-HT_6 receptor (pK_i = 7.8 and 7.9, respectively) and > 100-fold selectivity over other receptor sites. Furthermore, these compounds behaved as competitive antagonists, causing a parallel shift in the dose response curve to 5-HT, and had no effect on the basal level of cAMP, suggesting that they are antagonists at the 5-HT_6 receptor [4,38]. Ro 04-6790 was sufficiently brain penetrant that a dose of 30 mg/kg was predicted to occupy > 70% of 5-HT_6 receptors. Ro 63-0563 was radiolabelled with tritium in positions 3 and 5 of the benzene ring and used to label both the rat and human recombinant receptor systems [39]. Specific binding of [^{3}H]-Ro 63-0563 to recombinant rat and human 5-HT_6 receptor was saturable, rapid and reversible with respective equilibrium dissociation constant or K_d values of 6.8 and 4.96 nM. The pharmacological profile of both receptors radiolabelled with [^{3}H]-Ro 63-0563 was similar to that obtained with either [^{3}H]-LSD or [^{3}H]-5-HT. Recently, Bös *et al.* published the SAR within the Ro 04-6790 and Ro 63-0563 series. It has been shown that the ethyl group at the amino substituent 64, as well as small rings such as azetidine 65 and pyrrolidine 66 in position 2, gave compounds with similar 5-HT_6 receptor affinities compared to the lead compound 12. The introduction of larger groups or no substitution at the amino group in this position, led to ligands with reduced 5-HT_6 receptor affinity. It was claimed that omitting the 4-amino functionality of the lead compound 12 or replacing it by other substituents, such as halogen or alkyl groups, resulted in a loss of 5-HT_6 receptor affinity. Replacement of the pyrimidine derivative with a pyridine 13 increased binding affinity (pK_i = 7.8). The bromo substituted compound 67 showed a decrease in human 5-HT_6 receptor affinity, with a concomitant increase in compound lipophilicity (pK_i = 7.3 and logD = 1.7, compared to 7.8 and 0.03 for 13). Replacement of the heterocyclic nucleus with a simple phenyl ring produced ligands with high affinities for the 5-HT_6 receptor. For example, for the bromo-amino (68) and methoxy-amino (69) derivatives bind with affinities (pKi) of 7.7 and 7.8, respectively. Incorporation of the amino nitrogen of this series of ligands into a 4-sulfamoylsubstituted indole led to potent 5-HT_6 receptor antagonists, such as 4-(4-aminobenzylsulfonyl)-6-bromo-1H-indole 70 (pK_i = 7.3 and logD = 1.77) [40-42,107]. Similarly, in another patent application the same team claimed a series of novel pyrazolopyrimidine and pyrazolotriazine compounds with 5-HT_6 receptor affinity. These ligands were claimed to be suitable for the treatment and prevention of AD, Huntington's chorea, motor neuron disease, PD, psychosis, schizophrenia and depression. 3-(benzenesulfonyl)-5-methyl-2-(methylthio)pyrazolo[1,5-a]pyrimidin-7-amine (71) was one of the compounds specifically claimed. Standard assay methods were used to determine 5-HT_6 receptor binding affinity. The compounds exhibited pK_i values in the range 6.5 – 9.5. However, no specific data were disclosed [108].

Ro 04-6790 was the first potent, selective 5-HT_6 receptor antagonist used in behavioural studies [38,43]. As a confirmation of the *in vivo* activity of this compound, Woolley *et al.* recently published a study investigating the effect of intracerebroventricular administration of 5-HT_6 antisense oligonucleotide (5-HT_6 AO) complementary to bases 1 – 18 of the rat cDNA initiation sequence (1.5 mg b.i.d. for six days) and i.p. injection of Ro 04-6790 (10 or 30 mg/kg once-daily for three



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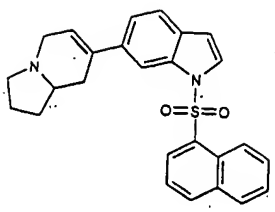

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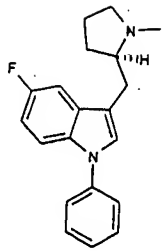

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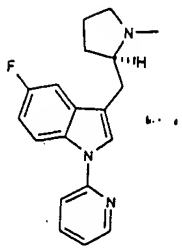

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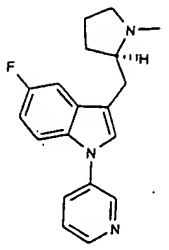

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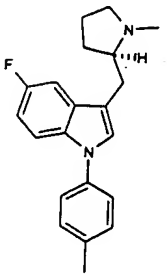

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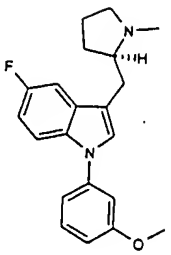

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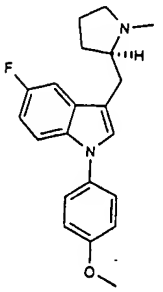

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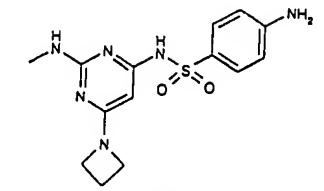
compound 32 was found to be a competitive antagonist (IC_{50} = 7.2 nM), with good selectivity over a number of other key serotonergic and dopaminergic receptors [32,105].

Recently, NPS researchers also published a series of 5-fluoro-(*R*)-3-(*N*-methypyrrolidin-2-yl-methyl)-1-arylindole derivatives as highly potent and selective human 5-HT_6 ligands. The SAR showed that, the simple phenyl analogue 40 was a promising initial lead giving a highly potent human 5-HT_6 receptor ligand with a K_i value of 2.7 nM and a selectivity of 52.5-fold versus human 5-HT_7 receptor. By comparison, the pyridyl analogues 41 and 42 gave greatly reduced potency and selectivity (K_i values of 295 – 147 nM, respectively, and 3.6 – 4.7-fold selectivity vs. 5-HT_7). In contrast, substitution on the phenyl ring was tolerated to varying degrees. Substitution with a single methyl residue provided compound 43 with an enhanced human 5-HT_6 receptor affinity (compared to 40) and good selectivity over the 5-HT_7 receptor. The methoxy residue in the *meta* position (44) or *para* position (45) retained 5-HT_6 receptor binding affinity. However, the concomitant reduction in 5-HT_7 receptor affinity resulted in an enhanced 5-HT_7/5-HT_6 selectivity (85.5 to 168-fold). The methoxy substituent at the *ortho* position was less favourable, with reduced potency and only moderate 5-HT_7/5-HT_6 selectivity. Substitution with fluoro at the *para* position resulted in enhanced binding (5-HT_6 = 0.34 nM) and good selectivity (5-HT_7/5-HT_6 = 70.5-fold) but *ortho* substitution decreased binding affinity (3-fold less potent than simple phenyl analogue 40). In contrast to the fluoro and methyl at *para* position analogues that resulted in improvements versus compound 40, the combination of the two groups in the trifluoromethyl analogues resulted in reduction of both potency and selectivity versus compound 40. The nitro group was not well-tolerated, giving results similar to the pyridyl analogues 41 and 42, with reduced potency and selectivity versus compound 40. Of the dimethyl analogues examined, only the 2,3-dimethyl analogue resulted in improved potency (5-HT_6 = 0.87 nM) and selectivity (5-HT_7/5-HT_6 = 131-fold) over 40. The symmetrical 3,5-dimethyl derivative was least well-tolerated, losing both potency (5-HT_6 = 165 nM) and selectivity (5-HT_7/5-HT_6 = 9.2-fold) [33]. Similarly, in collaboration with NPS Pharmaceuticals, Prof. Glennon *et al.* have described a series of N1-(benzenesulfonyl)tryptamine derivatives as novel human 5-HT_6 receptor antagonists. N1-Benzenesulfonamido-5-methoxy-*N*,*N*-dimethyltryptamine 46 binds to the human 5-HT_6 receptor with higher affinity (K_i = 2.9 ± 0.4 nM) than that of 5-HT (47, K_i = 78 ± 6 nM) itself. Replacement of the N_1-benzenesulfonamido group in compound 46 with the sterically larger 2-naphthalene-sulfonamido group 48, 1-naphthalenesulfonamido group 49 or 2,5-dimethoxyphenyl 50 also had little effect (K_i = 1.6 ± 0.3 nM and K_i = 0.9 ± 0.2 nM, respectively). Moving the 5-methoxy substituent of 46 to the 4-, 6- and 7-positions led to analogues 51, 52 and 53, respectively. With the exception of the 7-methoxy derivative 53 (K_i = 240 ± 32 nM, ~ 170-fold reduction in affinity), the 5-HT_6 affinity was decreased only by ~ 4-fold when compound 50 (K_i = 1.3 ± 0.2 nM) is compared with 51 (K_i = 7.4 ± 0.6 nM) and 52 (K_i = 9.5 ± 0.6 nM). Given the high affinity of compound 46 for the human 5-HT_6 receptor, the binding of this compound was examined at several other serotonin receptor populations and it was found to have > 100-fold selectivity over serotonin receptors, such as h5-HT_{1A}, h5-HT_{1B}, h5-HT_{1E}, h5-HT_3 and h5-HT_7. In contrast, 46 displayed high affinity for rat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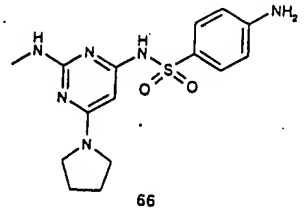
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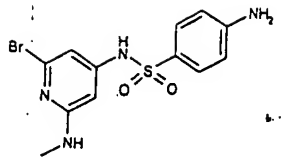
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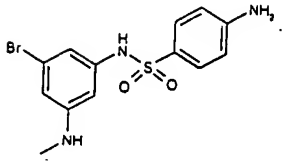
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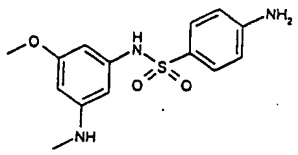
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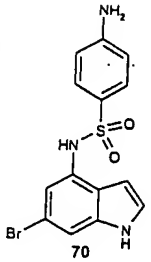
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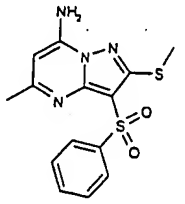
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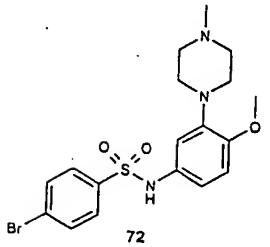
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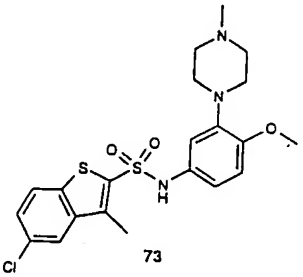
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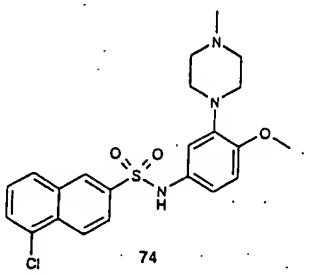
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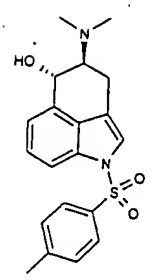
days) on acquisition and retention in the morris water maze. Neither the 5-HT$_6$ AO nor Ro 04-6790 affected acquisition, but both enhanced retention of the learned platform position such that the rat spent significantly longer searching the trained platform position than any other area during the probe tests. Furthermore, neither AO nor Ro 04-6790 had any effect on the time taken to reach a raised visible platform, indicating that visual acuity was unimpaired. In addition, antisense oligonucleotide reduced both food consumption and body weight and the later effect was also seen following Ro 04-6790, suggesting a role for the 5-HT$_6$ receptor in the regulation of feeding. Hence, while the underlying mechanism remains unclear, enhanced retention of spatial learning following both AO and 5-HT$_6$ receptor antagonist administration strongly indicates a role for this receptor in memory processes [44]. Furthermore, as mentioned above, i.p. administration of Ro 04-6790 produced a behavioural syndrome in rats consisting of yawing, stretching and chewing, which could be antagonised by atropine and scopolamine, suggesting a modulation of cholinergic mechanisms [41,45].

3.3 GlaxoSmithKline

High-throughput screening efforts from the SmithKline Beecham group (now GlaxoSmithKline) against the human 5-HT$_6$ receptor led to the identification of 4-bromo-*N*-[4-methoxy-3(4-methoxypiperazin-1-yl)phenyl]benzenesulfonamide 72 as the lead compound. Compound 72 showed excellent binding affinity for the 5-HT$_6$ receptor (pK_i = 8.3) and > 50-fold selectivity over a number of other key receptors, including 5-HT receptor subtypes. Furthermore, compound 72 was found to be a competitive antagonist in a functional model of 5-HT$_6$ receptor activation where, 5-HT-stimulated adenylyl cyclase activity in membranes from HeLa cells transfected with the human 5-HT$_6$ receptor (pK_b = 7.8 ± 0.2). The pharmacokinetic studies at steady-state in rats, following 8 h i.v. infusion demonstrated that compound 72 was moderately brain penetrant (25%), but was subject to rapid blood clearance (~ 60 ml/min/kg), resulting in low oral bioavailability (F = 12% via po. administration). The SARs around the aryl group of the sulfonamide moiety of the lead structure 72 yielded a range of affinities for the 5-HT$_6$ receptor with a number of analogues, such as derivatives 73 – 77. These compounds demonstrated improved binding profiles compared to the lead compound 72 (pK_i = 9.2, 9.1, 8.9, 8.6 and 8.5, respectively). On the other hand, the polar aryl groups, such as imidazole ring 78, showed very poor human 5-HT$_6$ receptor affinity (pK_i = 6.1) [46,109-113]. The replacement of the sulfonamide group by a benzamide moiety was the subject of another patent application from the same team. These compounds were claimed to be selective antagonists for the 5-HT$_6$ receptor. Compound N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-*N*-methyl-4-phenylbenza-



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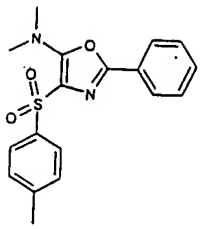

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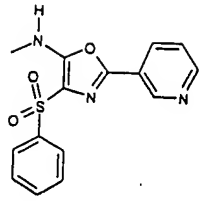

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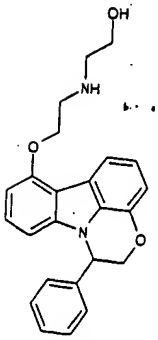

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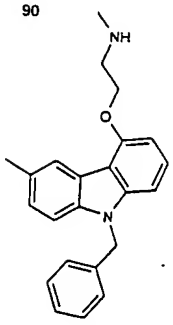

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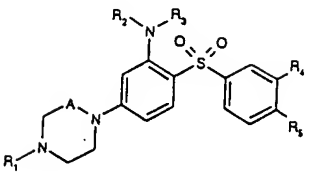

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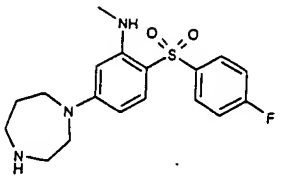

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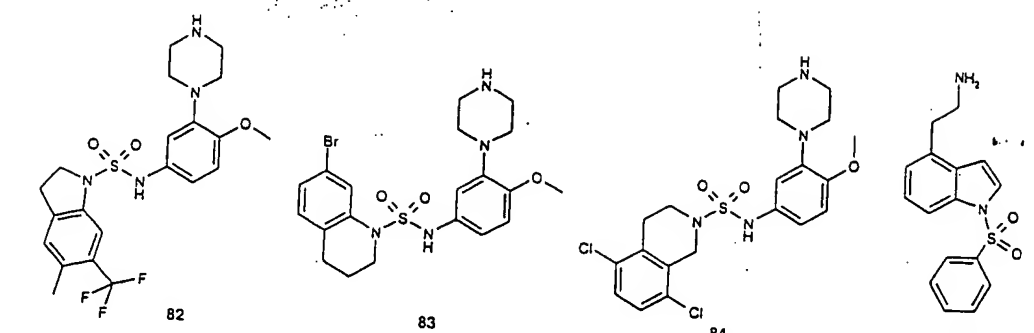
of those ligands. The binding affinities (K_i values) were in the range 1.1 - 97 nM. The specific compound 2-[2-(1-phenyl-1,2-dihydro[1,4]oxazino[2,3,4,jk]carbazol-7-yloxy)ethylamino]ethanol 93 is one of 22 compounds specifically claimed, having a K_i value of 1.1 nM [119]. The second patent application disclosed a family of aminoalkoxy carbazoles as 5-HT_6 receptor ligands. Similarly, the compounds were assessed for their *in vitro* 5-HT_6 receptor binding activity and showed K_i values ranging from 2.2 nM to 482 nM. The specific compound, N-[2-(9-benzyl-6-methyl-9H-carbazol-4-yloxy)-ethyl]-*N*-methylamine 94, was one of 62 analogues specifically claimed and had a K_i value of 2.2 nM [120].

During the preparation of this review, a third patent application was published from Pharmacia & Upjohn on a series of bis-arylsulfone derivatives exemplified by the general structure 95. These compounds were claimed to be useful for the treatment of diseases in which the 5-HT receptors, particularly the 5-HT_6 receptor, is implicated such as anxiety, depression, schizophrenia, obsessive/compulsive disorder, migraine, addiction, obesity, eating disorders, sleep disorders and numerous other CNS diseases. These compounds were stated to be 5-HT_6 receptor ligands, which selectively bind to the 5-HT_6 receptor. The specific compound 5-(1,4-diazepan-1-yl)-2(4-fluorophenylsulphonyl)-*N*-methylaniline 96 is one of 222 compounds specifically claimed. The K_i value for the corresponding hydrochloride was found to be 1.4 nM [121].

4. Expert opinion

The 5-HT_6 receptor was identified and characterised using molecular biological techniques and evidence is accumulating that this receptor mediates specific functions. Based on the aforementioned evidence, it is now becoming clear that targeting the 5-HT_6 receptor with selective antagonists is a viable drug development strategy, since novel drugs with potential for treating a large number of common disorders, including schizophrenia and cognitive dysfunction, are possible. Furthermore, the CNS specific localisation of this receptor makes this target very attractive for the treatment of CNS disorders with little likelihood of peripheral side effects. As a result, the identification of potent, selective and structurally diverse 5-HT_6 receptor antagonists, such as those described above, should give researchers confidence for investigating whether the observed pharmacological effects are due to 5-HT_6 receptor antagonism or to the properties of a particular compound. In addition, the growing number of novel selective 5-HT_6 receptor ligands from the different structural classes should also help in resolving the lack of agreement in previous animal experiments. The ongoing drug discovery efforts geared toward the development of novel 5-HT_6 receptor antagonists can potentially usher in a new generation of drugs with enhanced efficacy and reduced side effects. The development of these drugs may revolutionise the treatment of a number of common CNS disorders.



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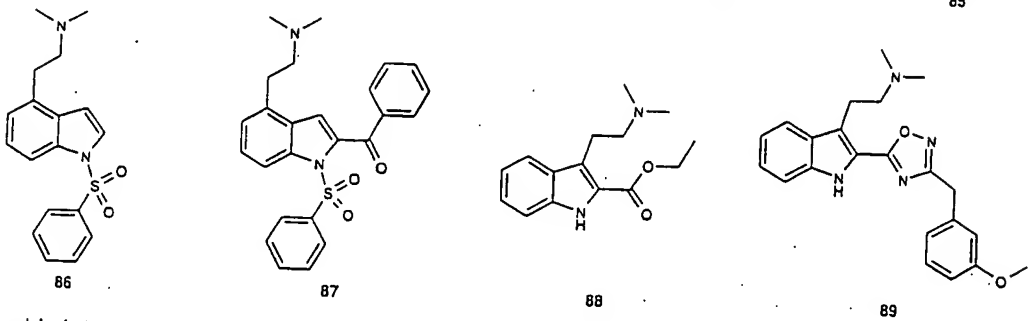

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model, derivative 46 dose-dependently reduced the mescaline-induced head twitches in rats when administered i.p., suggesting an *in vivo* CNS effect. In addition, pharmacokinetic analysis in rats showed the brain-plasma ratio of 7.4 at 30 min after i.p. administration of compound 46 (3 mg/kg). Further structure-activity variation within the indole sulfonyl series, led to the discovery of the 4-aminoethylindole analogues 85, 86 and 87, which displayed 5-HT$_6$ receptor binding affinity with K_i values of 2.4 nM, 1.5 nM and 3.0 nM respectively. However, subsequent functional analysis of compound 86 showed partial agonist behaviour. A large sterically demanding substituent in the 2-position of the indole core, such as a benzoyl group, was well-tolerated in the presence of the N-1 benzenesulphonyl groups, as illustrated by compound 87. It was previously discovered by NPS researchers that removal of the N-1 benzenesulphonyl group was detrimental to the 5-HT$_6$ receptor antagonist activity. Merck researchers showed that the introduction of an ethyl ester function in the 2-position of the dimethyltryptamine in the absence of N-1 substitution recovers both 5-HT$_6$ receptor affinity and antagonist activity (compound 88, K_i = 20 nM). Although this compound possessed reasonable human 5-HT$_6$ receptor affinity, it was not very selective over other serotonin and dopamine receptor subtypes. Further elaboration of the carboxy functionality to the 3-methoxybenzyloxadiazole derivative 89 led to a compound with high 5-HT$_6$ receptor affinity (K_i = 1.3 nM), but no selectivity data were reported. Finally, supporting evidence for the proposed binding conformation of the basic aminoethyl group was gained from the conformationally rigid 5-hydroxy-1, 3,4,5-tetrahydrobenz[cd]indole 90, which displayed good 5-HT$_6$ receptor affinity (K_i = 7.2 nM). In addition, compound 90 showed excellent selectivity over all the serotonin and dopamine receptors tested, with the lowest being against the 5-HT$_2$ receptor (44-fold selectivity). Two other Merck patent applications were published in June 2000 and May 2001, respectively, describing novel sulfonyloxazole-amine derivatives with affinity to the 5-HT$_6$ receptors. *In vitro* tests on the compounds indicated a selective affinity for 5-HT$_6$ receptors of < 4 nM and were claimed to be 5-HT$_6$ receptor agonists or antagonists. However, no specific data were presented. N,N-dimethyl-2-phenyl-4-(4-methylphenylsulfonyl)oxazol-5-yl-amine 91 and N-[4-phenylsulfonyl-2-(pyridin-3-yl)oxazol-5-yl]methylamine 92 were among the compounds specifically claimed [117,118].

3.5 Pharmacia & Upjohn

Two 5-HT$_6$ receptor patent applications appeared from the Pharmacia & Upjohn group (now Pharmacia) during 2001. One application claimed a series of novel oxazincarbazole compounds and a method for their use for the treatment of CNS diseases (including anxiety disorder, psychosis, schizophrenia and depression) by modulation of 5-HT$_6$ receptor function. An *in vitro* 5-HT$_6$ receptor binding assay using [^{3}H]-LSD on Hela cell membranes containing cloned human 5-HT$_6$ receptors was used to determine the potency

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